Thiazolidinone prodrugs activated by reactive oxygen species for use in the treatment of inflammatory diseases and cancer

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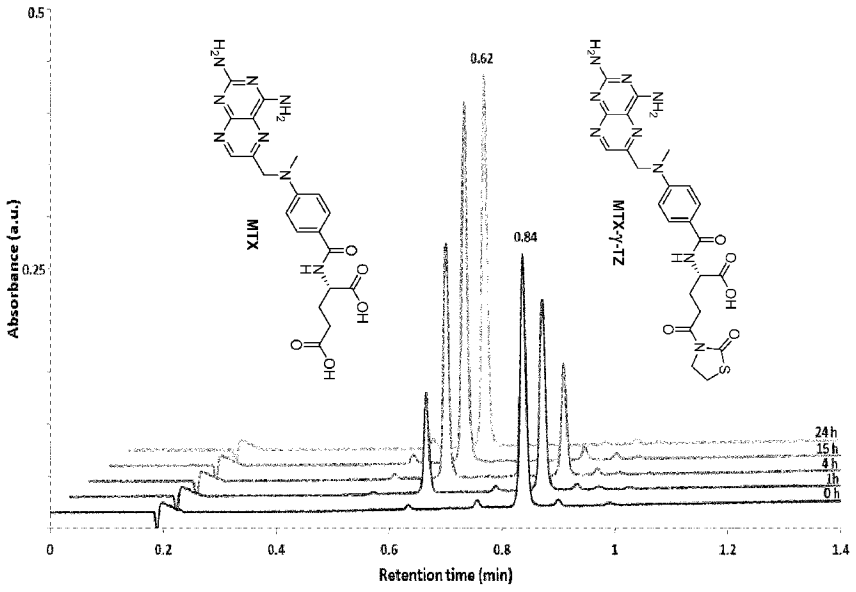
FIG. 1



(57) Abstract: Prodrugs activated predominantly or exclusively in inflammatory tissue, more particularly prodrugs of methotrexate and derivatives thereof, which are selectively activated by Reactive Oxygen Species (ROS) in inflammatory tissues associated with cancer and inflammatory diseases, as well as method for preparing said prodrugs.

[Continued on next page]

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PRODRUGS ACTIVATED BY REACTIVE OXYGEN SPECIES FOR USE IN THE TREATMENT OF INFLAMMATORY DISEASES AND CANCER

The present invention relates to prodrugs which are activated predominantly or exclusively in inflammatory tissue. More particularly, the present invention relates to prodrugs of methotrexate and derivatives thereof, which are selectively activated by Reactive Oxygen Species (ROS) in inflammatory tissues associated with cancer and inflammatory diseases such as rheumatoid arthritis (RA), juvenile dermatomyositis, psoriasis, psoriatic arthritis, lupus, sarcoidosis, Crohn's disease, colitis ulcerosa, multiple sclerosis, Amyotropic Lateral Sclerosis (ALS), atopic dermatitis, eczema etc.

BACKGROUND

The current therapies for the treatment of cancer and the palliation of symptoms in chronic inflammatory diseases such as rheumatoid arthritis (RA), employing chemotherapy and anti-inflammatory therapeutics, are well-known to produce severe side-effects due their side-effect profile and poor selectivity.

Prodrugs are masked forms of pharmacologically active agents designed to undergo *in vivo* activation by specific stimuli. By introduction of prodrug chemical moieties that makes the drug in question inactive in healthy tissue and selectively activated in diseased tissue the side-effect profile and the selectivity may be improved significantly.

The concentration of Reactive Oxygen Species (ROS) is increased in inflammatory tissues associated with cancer and rheumatoid arthritis compared to healthy tissue. This unique environment of the inflammatory tissue can therefore be used as a trigger stimulus and in turn enable more selective palliative treatment of diseases associated with chronic inflammation, as well as in cancer therapy, by reducing side-effects stemming from cross-reactivity with healthy tissue.

Methotrexate is an anti-cancer drug, a so-called anti-folate, which acts by inhibiting the metabolism of folic acid via dihydrofolate reductase. Methotrexate is also widely used as a disease-modifying treatment for some autoimmune diseases, including rheumatoid arthritis, juvenile dermatomyositis, psoriasis, psoriatic arthritis, lupus, sarcoidosis, and Crohn's disease.

US 2013/0045949 A1 related to prodrugs that are selectively activated to produce active anti-cancer agents in tumor cells using phenylboronates and phenylboronic acids as the trigger moiety.

US 2015/0005352 A1 discloses ROS-sensitive prodrug compositions and methods of treating ROS-associated diseases by administering the ROS-sensitive prodrug compositions.

WO 2012/123076 A1 relates to ferrocene-based compounds and their use as ROS-regulating prodrugs.

Xiaohua Peng & Varsha Gandhi, "ROS-activated anticancer prodrugs: a new strategy for tumor-specific damage", Therapeutic Delivery (2012), 3(7), 823-833 discloses the use of boronic acids/esters as triggers for developing ROS-activated anticancer prodrugs that target cancer cells.

Perez et al., "Exploring hydrogen peroxide responsive thiazolidinone-based prodrugs", Chem. Commun., 2015, 51, 7716-7119 disclose the use of thiazolidinone protecting groups for ROS sensitive prodrugs.

WEI WEN-HAO ET AL.: "Gadolinium texaphyrin-methotrexate conjugates. Towards improved cancer chemotherapeutic agents", ORGANIC & BIOMOLECULAR CHEMISTRY, ROYAL SOCIETY OF CHEMISTRY, GB, vol. 3, no. 18, 21 September 2005, p. 3290-3296 discloses methotrexate conjugates and their use.

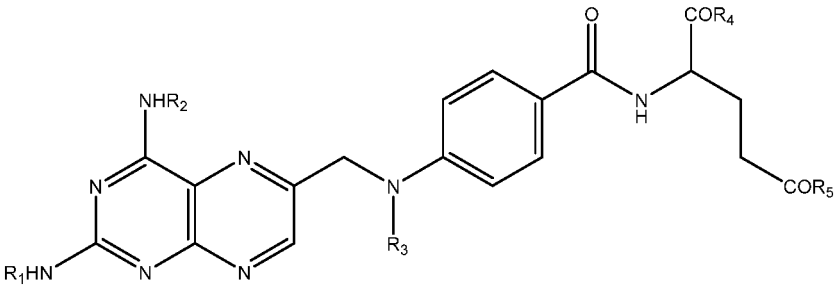
KHAN; Z.A. ET AL.: "Methotrexate: a detailed review on drug delivery and clinical spects", EXPERT OPINION ON DRUG DELIVERY, vol. 9, 2012, p. 151-169 describes methotrexate and uses thereof for the treatment of various types of malignancy, psoriasis, rheumatological diseases and the medical termination of pregnancy.

There is still a need for novel prodrugs of ROS-sensitive drug compositions, in particular prodrugs of methotrexate, which may be used for site-specific treatment, are stable, and lend themselves for up-scaling.

It is therefore an object of embodiments of the invention to provide prodrugs of ROS-sensitive drug compositions, in particular prodrugs of methotrexate, which are selectively activated in inflammatory tissues, have a beneficial cytotoxicity in target cells, low (or no) cytotoxicity in healthy cells, are stable, and have a satisfactory bioavailability at the intended site of action.

SUMMARY

A compound having the formula I is provided:

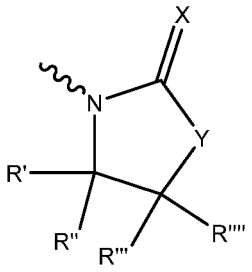


I

wherein R1 and R2 are hydrogen;

R3 is selected from the group consisting of hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, and C_{2-6}alkynyl;

R4 and R5 are selected from the group consisting of OH, O-C_{1-6}alkyl, O-C_{2-6}alkenyl, O-C_{6-12}aryl, O-C_{4-11}heteroaryl, O-C_{1-6}alkyl-C_{6-12}aryl, and a ring structure of formula II:

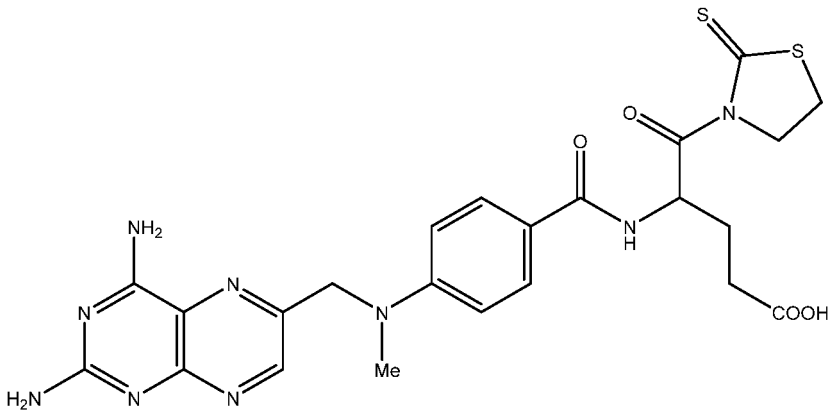


II

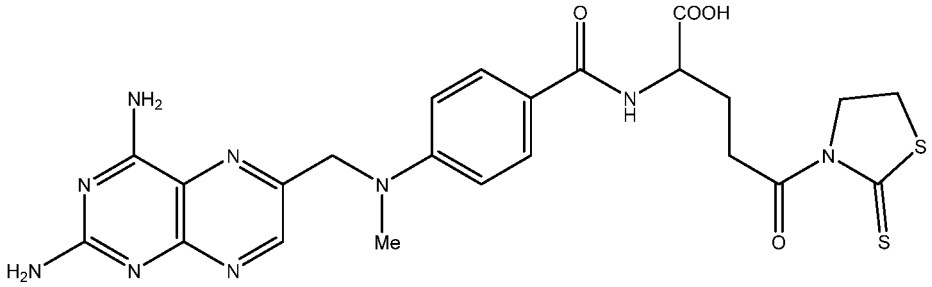
wherein X and Y are independently S or O, and R', R'', R''' and R'''' are independently selected from hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, C_{6-12}aryl, C_{4-11}heteroaryl and C_{1-6}alkyl-C_{6-12}aryl;

provided that at least one of R4 and R5 have the ring structure of formula II;

with the proviso that the compounds



and



are disclaimed.

Pharmaceutically acceptable salts, solvates, and stereoisomers of the compound of formula I are also provided. Methods for the preparation of said compounds, and their use in medical methods of treatment are also provided.

Pharmaceutical compositions comprising a compound of the formula I optionally in combination with one or more excipients are also provided.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows RP-UPLC-MS UV (λ = 306 nm) chromatograms of the activation of compound **30** (MTZ-γ-TZ, t_R 0.84 min);

FIGURE 2 shows MCF-7 *in vitro* cell viability assay incubated with methotrexate and compound **30** (MTZ-γ-TZ);

FIGURE 3 shows NCI-H460 *in vitro* cell viability assay incubated with methotrexate and compound **30** (MTZ-γ-TZ);

FIGURE 4 shows *In vitro* cell viability study of MCF-7 cells incubated for 48 h with 0.25, 0.062 and 0.015 µM concentrations of methotrexate and compound **30** (MTZ-γ-TZ);

FIGURE 5 shows *In vitro* cell viability study of NCI-H460 cells incubated for 48 h with 0.25, 0.062 and 0.015 µM concentrations of methotrexate and compound **30** (MTZ-γ-TZ);

FIGURE 6 shows suppression of CIA development in mice during treatment with methotrexate (**MTX**), and prodrug **30** (MTZ-γ-TZ); and

FIGURE 7 shows the general health of mice was evaluated three times per week during CIA as the average body weight in groups of animals (n = 8) tested with vehicle, **MTX**, and prodrug **30** (MTZ-γ-TZ).

DETAILED DISCLOSURE

Definitions

In the present context the term "alkyl" means a linear, cyclic or branched hydrocarbon group having 1 to 24 carbon atoms, such as methyl, ethyl, propyl, *iso*-propyl, cyclopropyl, butyl, *iso*-butyl, *tert*-butyl, cyclobutyl, pentyl, cyclopentyl, hexyl, and cyclohexyl.

In the present context the term "alkenyl" means a linear, cyclic or branched hydrocarbon groups having 2 to 24 carbon atoms, and comprising (at least) one unsaturated bond. Examples of alkenyl groups are vinyl, allyl, butenyl, pentenyl, hexenyl, heptenyl, octenyl, nonenyl and decaenyl. Preferred examples of alkenyl are vinyl, allyl, butenyl, especially allyl.

In the present context the term "alkynyl" means a linear, cyclic or branched hydrocarbon groups having 2 to 24 carbon atoms, and comprising (at least) one triple bond. Examples of alkynyl groups are acetylene, propynyl, butynyl, pentynyl, and hexynyl.

In the present context the term "aryl" refers to an unsaturated cyclic system. Aryl groups may comprise from 4-12 atoms, suitably from 6-8 atoms, most suitably 6 atoms. "Aryl" is preferably phenyl ($-C_6H_5$).

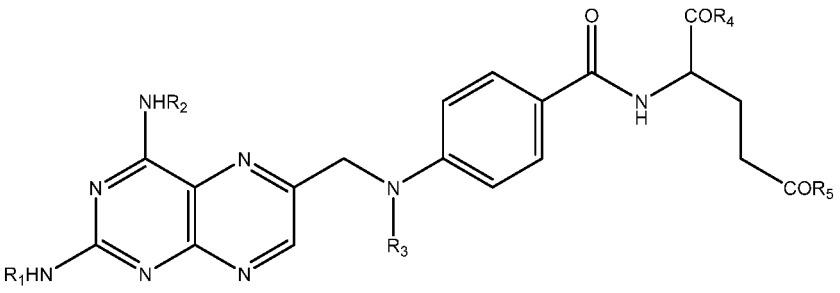
In the present context the term "heteroaryl" refers to an unsaturated cyclic system where one or more of the carbon atoms have been replaced with heteroatoms, e.g. nitrogen (=N- or –NH-), sulphur, and/or oxygen atoms. Heteroaryl groups may comprise from 4-12 atoms,

suitably from 5-9 atoms, such as 5-6 atoms, wherein at least one carbon atom has been replaced with a heteroatom, e.g. nitrogen (=N- or –NH-), sulphur, and/or oxygen atoms.

The term "pharmaceutically acceptable salt" is intended to indicate salts prepared by reacting a compound of formula I with a suitable inorganic or organic acid, such as hydrochloric, hydrobromic, hydroiodic, sulfuric, nitric, phosphoric, formic, acetic, 2,2-dichloroacetic, choline, adipic, ascorbic, L-aspartic, L-glutamic, galactaric, lactic, maleic, L-malic, phthalic, citric, propionic, benzoic, glutaric, gluconic, D-glucuronic, methanesulfonic, salicylic, succinic, malonic, tartaric, benzenesulfonic, ethane-1,2-disulfonic, 2-hydroxy ethanesulfonic acid, toluenesulfonic, sulfamic or fumaric acid. Pharmaceutically acceptable salts of compounds of formula I may also be prepared by reaction with a suitable base such as sodium hydroxide, potassium hydroxide, magnesium hydroxide, calcium hydroxide, ammonia, or suitable non-toxic amines, such as lower alkylamines, for example triethylamine, hydroxy-lower alkylamines, for example 2-hydroxyethylamine, bis-(2-hydroxyethyl)-amine, cycloalkylamines, for example dicyclohexylamine, or benzylamines, for example N,N´-dibenzylethylenediamine, and dibenzylamine, or L-arginine or L-lysine.

The term "solvate" is intended to indicate a species formed by interaction between a compound, e.g. a compound of formula I, and a solvent, e.g. alcohol, glycerol or water, wherein said species is in a solid form. When water is the solvent, said species is referred to as a hydrate.

As above, a compound having the formula I is provided:

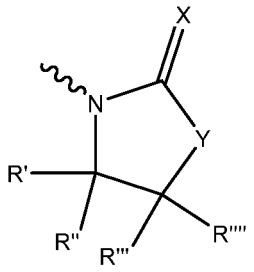


I

In formula I, R1 and R2 are hydrogen.

In formula I, R3 is selected from the group consisting of hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, and C_2-C_6alkynyl. R3 may be C_{1-6}alkyl, and is preferably C_{1-4}alkyl, more preferably methyl or ethyl, most preferably methyl.

In formula I, R4 and R5 are selected from the group consisting of OH, O-C_{1-6}alkyl, O-C_{2-6}alkenyl, O-C_{6-12}aryl, O-C_{4-11}heteroaryl, O-C_{1-6}alkyl-C_{6-12}aryl, and a ring structure of formula II:



II

In formula II, X and Y are independently S or O, and R', R'', R''' and R'''' are independently selected from hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, C_{6-12}aryl, C_{4-11}heteroaryl and C_{1-6}alkyl-C_{6-12}aryl. In one aspect, Y is S. In another aspect X is O. In a further aspect, R', R'', R''' and R'''' are hydrogen.

At least one of R4 and R5 has the ring structure of formula II. In one aspect, R4 is 3-thiazolidinonyl and R5 is OH or O-C_{1-6}alkyl. In another aspect, R4 is OH or O-C_{1-6}alkyl, and R5 is 3-thiazolidinonyl. In another aspect R4 and R5 are both 3-thiazolidinonyl.

Particular compounds of formula I are:

4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino)-N-(1,5-dioxo-1,5-bis(2-oxothiazolidin-3-yl)pentan-2-yl)benzamide (**28**),

tert-butyl (*S*)-2-(4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino)-benzamido)-5-oxo-5-(2-oxothiazolidin-3-yl)pentanoate (**67**), and

(*S*)-2-(4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino)benzamido)-5-oxo-5-(2-oxothiazolidin-3-yl)pentanoic acid (**30**, **MTZ-γ-TZ**).

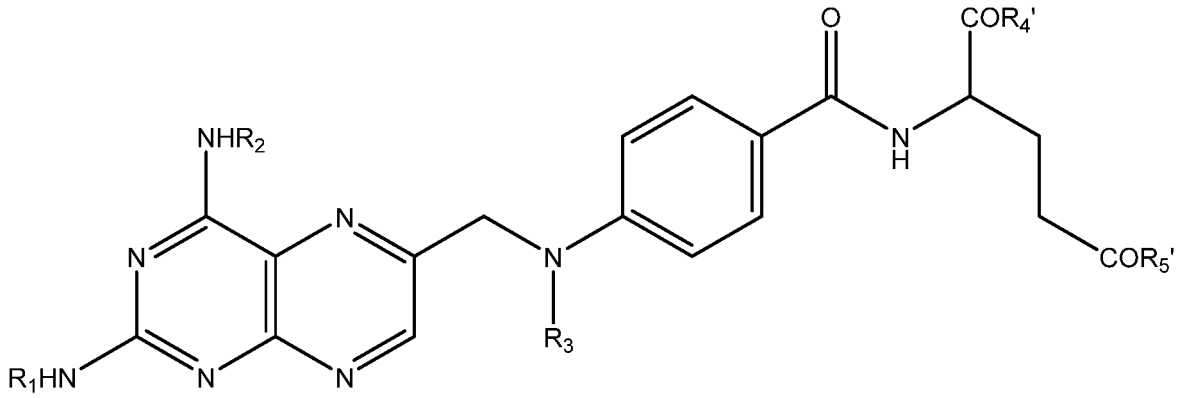
Pharmaceutically acceptable salts, solvates, and stereoisomers of the compound of formula I are also provided.

Compounds of formula I may comprise asymmetrically substituted (chiral) carbon atoms and carbon-carbon double bonds which may give rise to the existence of stereoisomeric forms,

e.g. enantiomers, diastereomers and geometric isomers. The present invention includes all such isomers, either in pure form or as mixtures thereof.

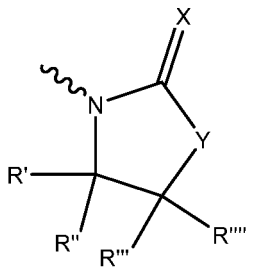
The compounds of formula I may be obtained in crystalline form either directly by concentration from an organic solvent or by crystallisation or recrystallisation from an organic solvent or mixture of said solvent and a cosolvent that may be organic or inorganic, such as water. The crystals may be isolated in essentially solvent-free form or as a solvate, such as a hydrate. The invention covers all crystalline modifications and forms and also mixtures thereof.

A method for the preparation of a compound of formula I is also provided. The method comprises the step of reacting a compound of formula IA,



IA

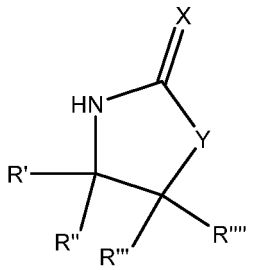
in which R1, R2, R3 are as defined above; and R4' and R5' are selected from the group consisting of OH, O-C_{1-6}alkyl, O-C_{2-6}alkenyl, O-C_{6-12}aryl, O-C_{4-11}heteroaryl, O-C_{1-6}alkyl-C_{6-12}aryl, and a ring structure of formula II:



II

wherein X and Y are independently S or O, and R', R'', R''' and R'''' are independently selected from hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, C_{6-12}aryl, C_{4-11}heteroaryl and C_{1-6}alkyl-C_{6-12}aryl; wherein at least one of R4' and R5' are OH;

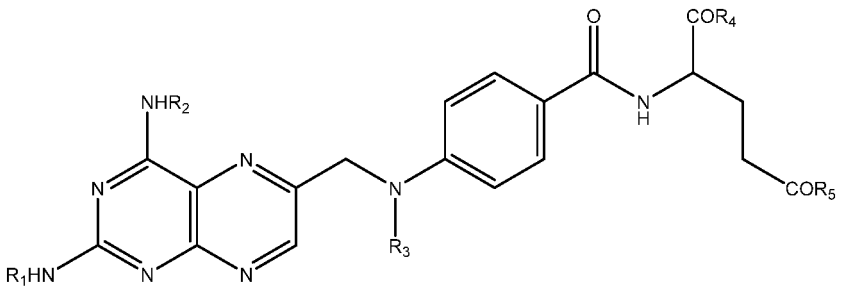
with a compound of formula IIA:



IIA

in which X, Y, R', R'', R''', and R'''' are as defined herein; in an amide coupling reaction. Optionally, a final deprotecton step is performed for synthesis of single thiazolidinone compounds containing a free carboxylic acid, e.g. compound **30** as illustrated below.

In another aspect the present invention relates to a pharmaceutical composition comprising a compound having the formula I

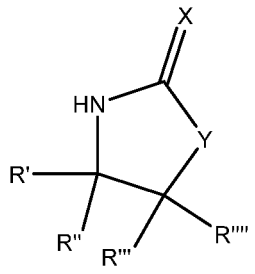


I

wherein R1 and R2 are hydrogen;

R3 is selected from the group consisting of hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, and C_2-C_6alkynyl;

R4 and R5 are selected from the group consisting of OH, O-C_{1-6}alkyl, O-C_{2-6}alkenyl, O-C_{6-12}aryl, O-C_{4-11}heteroaryl, O-C_{1-6}alkyl-C_{6-12}aryl, and a ring structure of formula II:



II

wherein X and Y are independently S or O, and R', R'', R''', and R'''' are independently selected from hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, C_{6-12}aryl, C_{4-11}heteroaryl and C_{1-6}alkyl-C_{6-12}aryl;

provided that at least one of R4 and R5 have the ring structure of formula II;

as well as pharmaceutically acceptable salts, solvates, and stereoisomers thereof, optionally in combination with one or more excipients.

The present invention further relates to a pharmaceutical composition comprising as an active ingredient an effective amount of at least one compound of the Formula I, or pharmaceutically acceptable salt thereof and/or stereoisomer thereof, optionally in combination with one or more conventional excipients. The pharmaceutical composition of the present invention usually comprises 0.1-90wt% of the compound of Formula I and/or physiologically acceptable salt thereof. The pharmaceutical composition can be prepared according to methods known in the art. For this purpose, if necessary, the compound of Formula I and/or a stereoisomer thereof is combined with one or more solid or liquid pharmaceutically acceptable excipients and/or adjuvants, to form an application form or dosage form suitable for administration to a human.

The compound of Formula I of the present invention or the pharmaceutical composition containing the same can be administered in unit dosage form, and the administration routes can be intestinal or parenteral administration, such as oral, intramuscular, subcutaneous, nasal, oral mucosal, skin, intraperitoneal or rectal administration. The administration dosage form can be, for example, tablets, capsules, drop pills, aerosols, pills, powders, solutions, suspensions, emulsions, granules, liposomes, transdermal agents, buccal tablets, suppositories, lyophilized powder injections, can be normal preparations, sustained-release

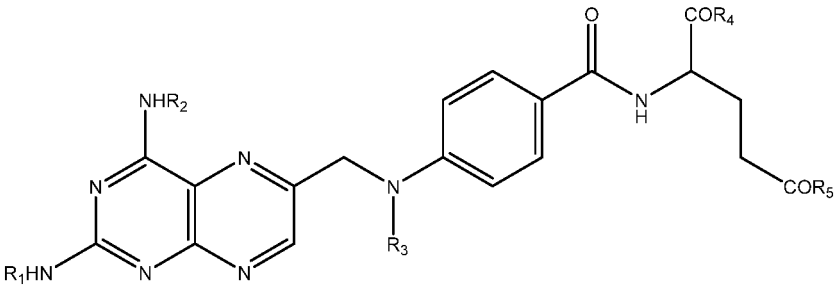
preparations, controlled-release preparations, and various microparticle administration systems. In order to process the unit dosage form into tablets, various carriers well known in the art can be widely used. The examples of the carriers can be, for example, diluents and absorbents, such as starch, dextrin, calcium sulfate, lactose, mannitol, sucrose, sodium chloride, glucose, urea, calcium carbonate, kaolin, microcrystalline cellulose, aluminum silicate; wetting agent and binding agent, such as water, glycerol, polyethylene glycol, ethanol, propanol, starch slurry, dextrin, syrup, honey, glucose solution, acacia mucilage, gelatin mucilage, sodium carboxymethylcellulose, shellac, methylcellulose, potassium phosphate, polyvinylpyrrolidone; disintegrants, such as, dry starch powder, alginate, agar powder, laminarin powder, sodium hydrogen carbonate and citric acid, calcium carbonate, polyoxyethylene sorbitol fatty acid ester, sodium dodecyl sulfate, methyl cellulose, ethyl cellulose; disintegration inhibitors, such as sucrose, tristearin, cocoa butter, hydrogenated oil; absorption enhancers, such as, quaternary ammonium salts, sodium dodecyl sulfate; lubricants, such as, talc, silica, maize powder, stearate, boric acid, liquid paraffin, polyethylene glycol. The tablets can be further processed into coated tablets, for example, sugar coated tablets, thin film coated tablets, enteric-coated tablets, or double-layer tablets or multi-layer tablets. In order to process the administration unit into pills, various carriers known in the art can be used. The examples of the carriers can be, for example, diluents and absorbing agents, such as glucose, lactose, starch, cocoa butter, hydrogenated vegetable oil, polyvinylpyrrolidone, Gelucire, kaolin, talc; binding agent, such as acacia gum, tragacanth gum, gelatin, ethanol, honey, liquid sugar, rice paste or panada; disintegrants, such as agar powder, dry starch powder, alginate, sodium dodecyl sulfonate, methyl cellulose, ethyl cellulose. In order to process the administration unit into suppositories, various carriers known in the art can be widely used. The examples of the carriers can be, for example, polyethylene glycol, lecithin, cocoa butter, fatty alcohol, ester of fatty alcohol, gelatin, semi-synthetic ester. In order to process the administration unit into capsules, the compound of Formula I or stereoisomer thereof as effective component is mixed with the various carriers, and the resultant mixture is placed in hard gelatin capsule shells or soft capsules. The compound of Formula I or stereoisomer thereof as effective component can also be processed into microcapsules, suspended in aqueous medium to form a suspension, or placed in hard capsules or processed into injections. In order to process the administration unit into a preparation for injection, such as solution, emulsion, lyophilized powder injection and suspension, all diluents known in the art, for example, water, ethanol, polyethylene glycol, 1,3-propylene glycol, ethoxylated isostearyl alcohol, multi-oxidized isostearyl alcohol, polyoxyethylene sorbitol fatty acid ester, could be used. In addition, in order to prepare an isotonic injection solution, an suitable amount of sodium chloride, glucose or glycerol can be added to the injection preparation, and conventional co-solvent, buffer agent, and pH regulator can further added.

In addition, if necessary, colouring agents, preservatives, flavoring agents, correctants, sweetening agents or other materials can also be added to the pharmaceutical compositions.

The administration dose of the compound of Formula I, or a stereoisomer thereof may depend on many factors, for example, the properties and severity of the diseases to be prevented or treated, the gender, age, bodyweight and individual reaction of patient or animal, the specific compound to be used, the administration routes and times, and so on. The dose can be of single dose form or can be divided into several dose forms, such as, two, three or four dose forms.

The compound according to formula I, and pharmaceutical compositions thereof may be used for the treatment of inflammatory diseases or cancer.

Thus, in a further aspect the present invention relates to a compound having the formula I

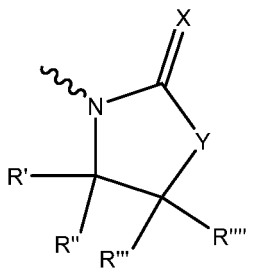


I

wherein R1 and R2 are hydrogen;

R3 is selected from the group consisting of hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, and C_2-C_6alkynyl;

R4 and R5 are selected from the group consisting of OH, O-C_{1-6}alkyl, O-C_{2-6}alkenyl, O-C_{6-12}aryl, O-C_{4-11}heteroaryl, O-C_{1-6}alkyl-C_{6-12}aryl, and a ring structure of formula II:



II

wherein X and Y are independently S or O, and R', R'', R''', and R'''' are independently selected from hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, C_{6-12}aryl, C_{4-11}heteroaryl and C_{1-6}alkyl-C_{6-12}aryl;

provided that at least one of R4 and R5 have the ring structure of formula II; as well as pharmaceutically acceptable salts, solvates, and stereoisomers thereof for use for the treatment of inflammatory diseases or cancer.

Non-limiting examples of inflammatory diseases include rheumatoid arthritis (RA), juvenile dermatomyositis, psoriasis, psoriatic arthritis, lupus, sarcoidosis, atopic dermatitis, eczema, Crohn's disease, colitis ulcerosa, multiple sclerosis, and Amyotropic Lateral Sclerosis (ALS). Non-limiting examples of cancer diseases include acute lymphocytic leukemia, meningeal leukemia, myeloproliferative neoplasm, breast cancer, squamous cell carcinoma, lymphosarcoma, osteosarcoma, advanced mycosis fungoides (cutaneous T cell lymphoma), small cell types lung cancer, non-small cell lung cancer, and non-Hodgkin's lymphoma.

A method of treatment of a patient suffering from inflammatory diseases or cancer is also provided, said method comprising administering a compound according to formula I to said patient.

General methods:

Commercially available reagents were used without further purification and all solvents were of HPLC quality. All reactions were run under a N_2 atmosphere and were monitored by thin layer chromatography (TLC) and/or reversed-phase ultra-performance liquid chromatography mass spectrometry (RP-UPLC-MS).

Analytical TLC was conducted on Merck aluminium sheets covered with silica (C60). The plates were either visualized under UV-light or stained by dipping in a developing agent followed by heating. $KMnO_4$ [3 g in water (300 mL) along with K_2CO_3 (20 g) and 5% aqueous

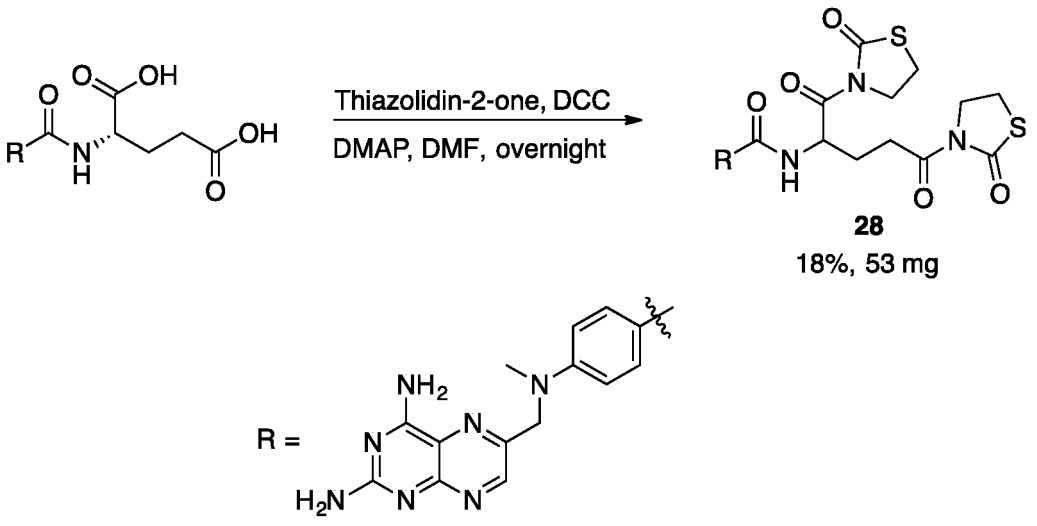
NaOH (5 mL)] and cerium molybdate [$Ce(NH_4)_2(NO_3)_6$ (0.5g), $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (24.0 g), and H_2SO_4 (24.0 g)] were used as developing agents. Flash column chromatography was performed using Merck Geduran® Si 60 (40-63 µm) silicagel.

All new compounds were characterized by ^1H NMR, ^{13}C NMR, IR, MS (ESI), HRMS (ESI), melting point, and optical rotation. For the recording of ^1H NMR and ^{13}C NMR a Bruker Ascend with a Prodigy cryoprobe (operating at 400 MHz for proton and 100 MHz for carbon) was used. The chemical shifts (δ) are reported in parts per million (ppm) and the coupling constants (*J*) in Hz. For spectra recorded in DMSO-d_6, signal positions were measured relative to the signal for DMSO (δ 2.50 ppm for ^1H NMR and δ 39.43 ppm for ^{13}C NMR). For spectra recorded in $CDCl_3$, signal positions were measured relative to the signal for $CHCl_3$ (δ 7.26 ppm for ^1H NMR and δ 77.0 ppm for ^{13}C NMR). IR analysis was performed on a Bruker Alpha FT-IR spectrometer. Analytical RP-UPLC-MS (ESI) analysis was performed on a S2 Waters AQUITY RP-UPLC system equipped with a diode array detector using an Thermo Accucore C18 column (d 2.6 µm, 2.1 x 50 mm; column temp: 50 °C; flow: 1.0 mL/min). Eluents A (0.1% HCO_2H in H_2O) and B (0.1% HCO_2H in MeCN) were used in a linear gradient (5% B to 100% B) in 2.4 min and then held for 0.1 min at 100% B (total run time: 2.6 min). The LC system was coupled to a SQD mass spectrometer. Analytical LC-HRMS (ESI) analysis was performed on an Agilent 1100 RP-LC system equipped with a diode array detector using a Phenomenex Luna C18 column (d 3 µm, 2.1 x 50 mm; column temp: 40 °C; flow: 0.4 mL/min). Eluents A (0.1% HCO_2H in H_2O) and B (0.1% HCO_2H in MeCN) were used in a linear gradient (20% B to 100% B) in a total run time of 15 min. The LC system was coupled to a Micromass LCT orthogonal time-of-flight mass spectrometer equipped with a Lock Mass probe operating in positive electrospray mode. Optical rotation was carried out using a Perkin-Elmer polarimeter 341. The temperature for all recordings was approximately 20 °C. Melting points were obtained using a Stuart SMP30 melting point apparatus.

Preparative RP-HPLC was carried out on a Waters Alliance reversed-phase HPLC system consisting of a Waters 2545 Binary Gradient Module equipped with either an xBridge BEH C18 OBD Prep Column (130 Å, 5 µm, 30 x 150 mm) or an xBridge Peptide BEH C18 OBD Prep Column (130 Å, 5 µm, 19 mm x 100 mm) both operating at 20 °C and a flow rate of 20 mL/min, a Waters Photodiode Array Detector (detecting at 210-600 nm), a Waters UV Fraction Manager, and a Waters 2767 Sample Manager. Eluents A1 (0.1% HCO_2H in H_2O) and B1 (0.1% HCO_2H in MeCN) or A2 (5 mM NH_4OAc in H2O) and B2 (5 mM NH_4OAc in MeCN) were used in the following gradient: 5% B to 70 % B in 10 min, hold for 3.5 min, then 70% B to 100% B in 1.5 min, and hold 3 minutes (total run time: 20 min).

EXAMPLE 1

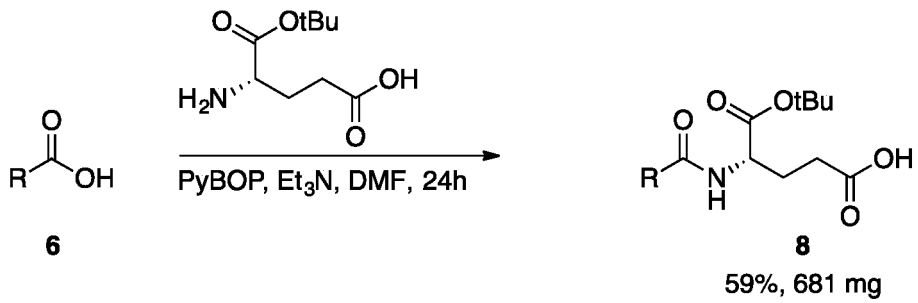
Synthesis of 4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino)-N-(1,5-dioxo-1,5-bis(2-oxothiazolidin-3-yl)pentan-2-yl)benzamide (**28**)

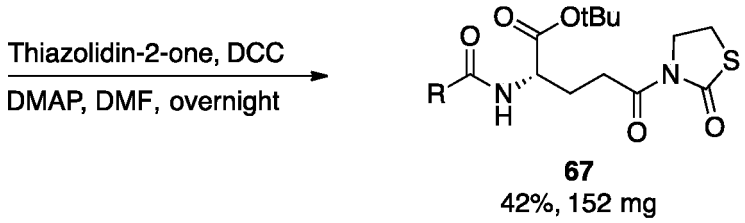


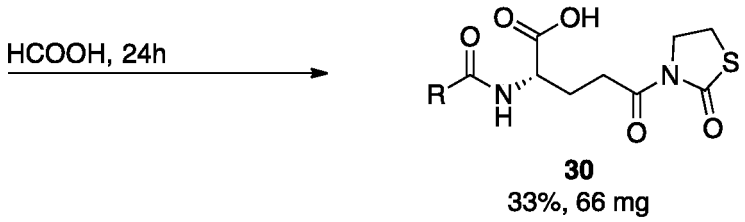
Synthesis scheme I

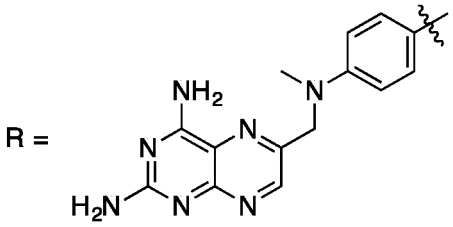
To a solution of methotrexate (209 mg, 0.460 mmol) in anhydrous DMF (5 mL), DCC (209 mg, 1.01 mmol) and DMAP (124 mg, 1.01 mmol) were added and the turbid reaction mixture was stirred for 20 min at 20 °C. Thiazolidin-2-one (95.1 mg, 0.922 mmol) was then added and the turbid mixture was stirred for 16 h. Precipitate was removed by filtration and the filtrate was purified by preparative HPLC (eluent system 1) to give the title compound as a yellow solid (53.1 mg, 18%). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.57 (s, 1H), 8.25 (d, J = 7.3 Hz, 1H), 7.72 (d, J = 8.9 Hz, 2H), 7.65 (br. s, 1H), 7.43 (br. s, 1H), 6.82 (d, J = 9.0 Hz, 2H), 6.61 (br. s, 2H), 5.28 (q, J = 7.0 Hz, 1H), 4.78 (s, 2H), 4.14 – 3.98 (m, 4H), 3.40 (t, J = 7.3 Hz, 2H), 3.33 (td, J = 7.2, 2.5 Hz, 2H), 3.21 (s, 3H), 2.98 (dt, J = 17.6, 7.5 Hz, 1H), 2.81 (dt, J = 17.6, 7.6 Hz, 1H), 1.98 (q, J = 7.2 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d6) δ 173.4, 173.2, 172.8, 172.0, 167.0, 163.4, 163.2, 155.6, 151.5, 149.6, 146.4, 129.5, 121.2 (2C), 111.5, 55.3, 52.8, 47.7, 47.4, 33.9, 25.7, 25.6, 25.3. HRMS (ESI) m/z calcd. for $C_{26}H_{29}N_{10}O_5S_2$ $[M+H]^+$ 625.1758, found 625.1779. M.p.: > 151 °C (decomposition); IR (neat) cm^{-1}: 3344, 3325, 3066, 3034, 2953, 2924, 1702, 1644, 1236, 1153; $[\alpha]_D^{20}$ = +1.8° (*c* 7.25 mg/mL in DMSO); purity by RP-UPLC-MS (PDA detector): > 95%.

EXAMPLE 2





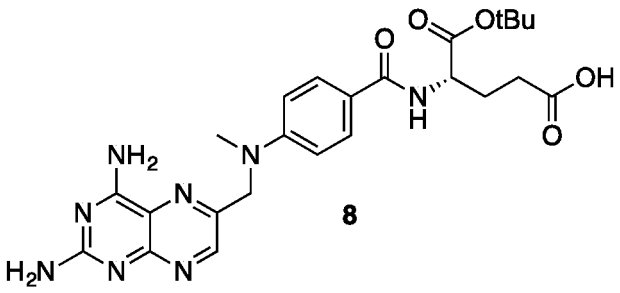




Synthesis of compounds **8**, **67**, and **30**.

2b

Synthesis of (*S*)-5-(*tert*-butoxy)-4-(4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino)-benzamido)-5-oxopentanoic acid (**8**)

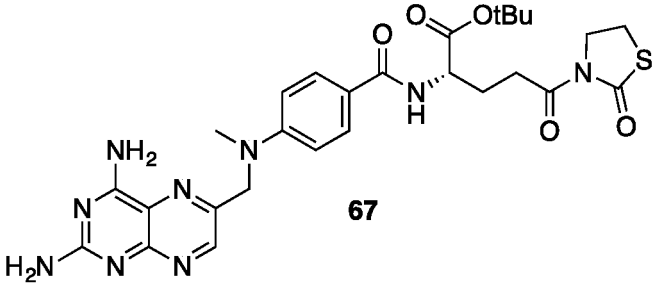


8

To a solution of pteroic acid **6** (771 mg, 2.37 mmol, 95% purity) in anhydrous DMF (20 mL), PyBOP (1.55 g, 2.98 mmol) and triethylamine (2.52 mL, 19.0 mmol) were added and the turbid reaction mixture was stirred 2 h at 20 °C. H-Glu-O*t*Bu was added and the turbid mixture was stirred for another 22 h. Then, 0.5 M NaOH (20 mL) was added and the aqueous phase was washed with a solution of CH_2Cl_2 and EtOAc (1:4, 1 x 50 mL) then neutralized with 1 M HCl and concentrated. The crude was taken up in DMF (20 mL) and precipitate was removed by filtration. The filtrate was purified by preparative HPLC (eluent system 1) to give the title compound as an orange solid (681 mg, 59%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.17 (br. s, 1H), 9.24 (br. s, 1H), 9.04 (br. s, 1H), 8.72 (s, 1H), 8.56 (br. s, 1H), 8.21 (d, *J* = 7.6 Hz, 1H), 7.74 (d, *J* = 9.0 Hz, 2H), 7.35 (br. s, 1H), 6.82 (d, *J* = 9.0 Hz, 2H), 4.87 (s, 2H), 4.29 (ddd, *J* = 9.7, 7.5, 5.2 Hz, 1H), 3.25 (s, 3H), 2.32 (t, *J* = 7.5 Hz, 2H), 2.06 – 1.96 (m, 1H), 1.90 (ddt, *J* = 14.1, 9.3, 7.2 Hz, 1H), 1.39 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.9, 171.5, 166.4, 162.8 (2C), 155.9, 151.2, 150.7, 148.8, 129.0, 122.3, 121.4, 111.2, 80.5, 54.9, 52.5, 40.2, 30.4, 27.7, 26.0; m.p.: > 154 °C (decomposition); IR (neat) cm^{-1}: 3342, 3118, 2976, 2931, 1716, 1639, 1601, 1506, 1364, 1152, 832.

2c

Synthesis of *tert*-butyl (*S*)-2-(4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino)-benzamido)-5-oxo-5-(2-oxothiazolidin-3-yl)pentanoate (**67**)

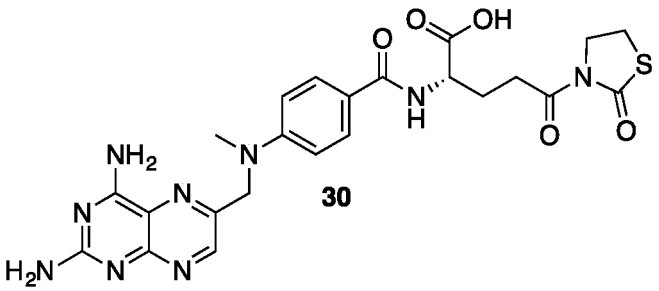


67

To a solution of MTX-α-O*t*Bu (**8**) (346 mg, 0.678 mmol, 90% purity) in anhydrous DMF (9 mL), DCC (283 mg, 1.37 mmol) and DMAP (333 mg, 2.73 mmol) were added and the turbid reaction mixture was stirred for 30 min at 20 °C. Thiazolidin-2-one (141 mg, 1.37 mmol) was then added and the turbid mixture was stirred for 16 h. Precipitate was removed by filtration and the filtrate was purified by preparative HPLC (eluent system 1) to give the title compound as a yellow solid (153 mg, 42%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (s, 1H), 8.18 (d, *J* = 7.5 Hz, 1H), 8.13 (s, 1H), 7.77 (br. s, 1H), 7.71 (d, *J* = 9.0 Hz, 2H), 7.56 (br. s, 1H), 6.82 (d, *J* = 9.0 Hz, 2H), 6.71 (br. s, 2H), 4.79 (s, 2H), 4.27 (ddd, *J* = 9.5, 7.5, 5.6 Hz, 1H), 4.04 (t, *J* = 7.3 Hz, 2H), 3.32 (td, *J* = 7.2, 1.7 Hz, 3H), 3.21 (s, 3H), 2.88 (td, *J* = 7.4, 2.4 Hz, 2H), 2.16 – 2.01 (m, 1H), 2.00 – 1.88 (m, 1H), 1.39 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.2, 172.1, 171.9, 166.8, 163.5, 163.2, 154.9, 151.4, 149.6, 146.8, 129.4, 121.9, 121.5, 111.5, 80.9, 55.3, 52.9, 47.4, 40.5, 33.2, 28.1, 25.8, 25.3. HRMS (ESI) *m/z* calcd. for $C_{27}H_{34}N_9O_5S$ $[M+H]^+$ 596.2398, found 596.2397; m.p.: > 142 °C (decomposition); IR (neat) cm^{-1}: 3325, 3183, 3116, 2974, 2924, 1635, 1604, 1506, 1446, 1362, 1151; $[\alpha]_D^{20}$ = -0.7° (*c* 7.14 mg/mL in DMSO); purity by RP-UPLC-MS (PDA detector): > 95%.

2d

Synthesis of (*S*)-2-(4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino)benzamido)-5-oxo-5-(2-oxothiazolidin-3-yl)pentanoic acid (**30**, **MTZ-γ-TZ**)



30

MTX-α-O*t*Bu-γ-TZ (**67**) (220 mg, 0.369 mmol) was dissolved in 100% formic acid (8 mL) and stirred for 24 h at 20 °C. The reaction mixture was concentrated *in vacuo* and the crude was dissolved up in DMF (2 mL) and purified by preparative HPLC (eluent system 1) to give the title compound as a yellow solid (66 mg, 33%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.57 (br. s, 1H), 8.57 (s, 1H), 8.18 (d, *J* = 7.7 Hz, 1H), 7.71 (d, *J* = 8.9 Hz, 2H), 7.65 (br. s, 1H), 7.44

(br. s, 1H), 6.82 (d, *J* = 9.0 Hz, 2H), 6.61 (br. s, 2H), 4.78 (s, 2H), 4.34 (ddd, *J* = 9.9, 7.6, 4.9 Hz, 1H), 4.13 – 3.96 (m, 2H), 3.32 (dt, *J* = 7.3, 1.9 Hz, 2H), 3.20 (s, 3H), 2.97 – 2.79 (m, 2H), 2.19 – 2.05 (m, 1H), 1.95 (dddd, *J* = 13.8, 9.7, 8.0, 5.8 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.3, 173.2, 172.2, 166.8, 163.6, 163.3, 155.6, 151.4, 149.7, 146.5, 129.4, 121.9, 121.6, 111.5, 55.3, 52.1, 47.5, 40.7, 33.4, 25.9, 25.3. HRMS (ESI) *m/z* calcd. for $C_{23}H_{26}N_9O_5S$ $[M+H]^+$ 540.1772, found 540.1779; m.p.: > 184 °C (decomposition); IR(neat) cm^{-1}: 3325, 3137, 3117, 2923, 2853, 1690, 1631, 1604, 1502, 1443, 1362, 1149; $[\alpha]_D^{20}$ = -8.0° (*c* 4.75 mg/mL in DMSO); purity by RP-UPLC-MS (PDA detector): > 95%.

EXAMPLE 3

Phosphate buffered saline stability

A 100 μg/mL stock solution of tested compound in 37 °C PBS was prepared. In triplicates, stock solution (1 mL) was incubated in Eppendorf tubes (1.5 mL) at 37 °C and 1000 rpm (Eppendorf Thermomixer C, 1.5 mL). Aliquots (80 μL) were collected at 0 h, 1 h, 2 h, 4 h, 8 h, and 24 h and analyzed directly by UPLC-MS.

Simulated gastric fluid stability

SGF (Sigma) was prepared according to manufacture specifications (0.5 mL conc. SGF solution in 12.5 mL milliQ water) and incubated Eppendorf tubes (1.5 mL) at 37 °C and 1000 rpm for 10 min (Eppendorf Thermomixer C, 1.5 mL). A 2 mg/mL stock solution of tested compound was prepared. In triplicates, pre-incubated SGF (0.95 mL) was added to stock solution (50 μL) and incubated at 37 °C and 1000 rpm. Aliquots (80 μL) were collected at 0 min, 15 min, 30 min, 45 min, 1 h, 1.5 h, 2 h, 3h, 4 h, and 24 h and analyzed directly by UPLC-MS

Kinetic solubility

Briefly, the kinetic solubility, utilizing test compound from 10 mM DMSO stock solution, is measured at a final compound concentration of 100 μM and 1% DMSO. Test compound is added to 100 mM potassium phosphate buffer, pH 7.4, and incubated at 37 °C for 20 hours in a heater-shaker (V 2000 heater shaker, Kisker-Biotech). After incubation, the samples are centrifuged at 3000xg at 37 °C for 30 min. (Centrifuge Model 5810R, Eppendorf) to pellet insoluble material and an aliquot of the supernatant is taken for analysis. After dilution of the sample, the concentration of dissolved compound is quantified by LC-MS/MS analysis.

Plasma protein binding assay

Briefly, the fraction unbound drug (f_u) in plasma from human or other animal species was determined by equilibrium dialysis at 37 °C for 4 hours using a Rapid Equilibrium Dialysis (RED) device (RED apparatus: Pierce RED Device, Nordic Biolabs). The drug molecule at a concentration of 10 μM is added to 50% plasma and dialyzed against isotonic phosphate buffer (67 mM, pH 7.4). After dialysis, the drug concentration in the buffer and plasma is quantified by LC-MS/MS analysis. In parallel, the stability of the drug molecule in plasma is determined by incubating drug-spiked plasma (10 μM) at 37 °C for 4 hours, meanwhile the control plasma sample is kept in the freezer. The concentration of drug in both samples is quantified by LC-MS/MS analysis.

Pooled human plasma originating from healthy donors, 1 male and 1 female (non-smoking), was obtained from Uppsala Academic Hospital. Citrate was used as anticoagulant. CD1-1 Mouse plasma K2 EDTA was obtained from Innovative Research Inc. Plasma was stored frozen in aliquots to avoid repeated freeze-thaw cycles. 50% plasma was made by thawing human or animal plasma at rt. and mixing it with a equal volume of isotonic phosphate buffer.

Metabolic stability

Briefly, the *in vitro* metabolic stability assay uses liver microsomes. Compound is dissolved in 100 mM KPO_4 buffer pH 7.4 to a 1μM final concentration. The assay is initiated by addition of NADPH and incubated for up to 40 min. (THERMOstar, BMG Lab Technologies) with microsomes. Samples are terminated at different time points by addition of acetonitrile. The amount of parent compound remaining is analyzed by LC-MS/MS. The natural logarithm of relative amount parent compound remaining is plotted against time and the first-order rate constant of consumption is determined by linear regression.

Liver microsomal fractions was purchased from XenoTech LLC, KS, USA: Pooled human liver microsomes (mixed gender), cat.no. H0610 and mouse (CD-1) liver microsomes, male cat.no. M1000

Table 1: Pharmacokinetic values for compound **30**.

	Kin. sol. (μM)	M. plasma stab. (%)[a,b]	M. %f_u[b]	H. plasma stab. (%)[a,b]	H. %f_u[b]
Comp. **30**	40	15	7	106	3

Comp.	PBS stab. $t_{1/2}$ (h)[b]	SGF stab. $t_{1/2}$ (h)[b]	M. metabolic stability CL_{int} (mL/min/kg)[b,c]	H. metabolic stability CL_{int} (mL/min/kg)[b,c]
30	36 ± 5	74 ± 13	33.13	4.43

[a] % compound recovered after 4 hours, [b] pH = 7.4, 37 °C, [c] using human microsomes
Kin. = kinetic, CL_{int}. = intrinsic clearance, Sol. = solubility, stab. = stability, M. = mouse, H. = human; $t_{1/2}$ = half-life, PBS = phosphate buffered saline, SGF = simulated gastric fluid

EXAMPLE 4

Cellular Assays

Cell Culture. The human breast cancer MCF-7 (Sigma) and human large cell lung cancer NCIH-460 (ATTC) cell lines were cultured in a humidified, 5% CO_2 atmosphere at 37 °C in Dulbecco´s Modified Eagle´s Medium (DMEM) (Sigma) or Roswell Park Memorial Institute medium (RPMI) 1640 (Sigma) supplemented with 10% fetal bovine serum (FBS, heat-inactivated, Fisher Scientific) and 1% penicillin/streptomycin. Both cell lines were subcultured every 2-3 days.

Pre-activation of compounds with H_2O_2. A 125 μM solution of tested compound was prepared in a 1.25 mM H_2O_2 in DMSO:PBS (1:1), placed in an Eppendorf tube and shaken at 21 °C for 24 h at 1000 rpm in an Eppendorf Thermomixer C (1.5 mL). The activation was followed by RP-UPLC-MS (λ = 306 nm) after 0 min, 15 min, 1 h, 4 h, and 24 h. A negative control consisting of a 125 μM solution of compound in a mixture of DMSO:PBS (1:1) without H_2O_2 was run in parallel to the activation assay in the same conditions.

Evaluation of cell viability. The 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-te-trazolium (MTS) assay (Promega Biotech AB, Stockholm, Sweden) was used to determine the in vitro antiproliferative effect of the compounds. This assay is based on the principle that cells have the ability to reduce MTS tetrazolium, while, when dead, they lose this ability. MCF-7 and NCI-H460 cells were cultured in 96-well plates at an initial density of 10^4 cells/well in their respective growth medium. After 24 h incubation to

allow cell attachment, the medium was removed and the cells were incubated in the presence or absence of pre-activated compounds at different concentrations. After 48 h incubation time, the MTS reagent was added to each well. The cells were further incubated for a period of time between 30-60 min at 37 ºC until colorimetric reaction was developed within the linear range and the absorbance of the samples was measured at 490 nm using a 96-well plate spectrophotometer (Victor 3 plate reader with Wallac 1420 Workstation vs 3.0 software).

A control was used for each tested compound, where cells were incubated with DMEM or RPMI containing the equivalent concentration of DMSO (maximum of 0.4% v/v). Each concentration of tested compounds was done in triplicates. The final concentration of H2O2 in each well was always <10 µM (non-cytotoxic concentration in MCF-7 and NCI-H460 cell lines, determined with the described assay). The IC50 values were calculated using GraphPad Prism v6.0 (California, USA) as the concentration of the compounds required to cause 50% response compared to cells exposed to controls using a non-linear dose-response regression.

Compound 30 according to the invention is indicated as "MTZ-γ-TZ**".**

Table 2: Evaluation of Cytotoxicity of MTZ-γ-TZ (**30**) and methotrexate by MTS assay.[a]

	IC_{50} (nM)[b]	
cell line	**MTX**	**MTX-γ-TZ**
MCF-7[a]	50 ± 24	40 ± 14
NCIH-460[a]	159 ± 77	92 ± 32

[a] MCF-7 and NCI-H460 cells were incubated with tested compounds over 48 h, and their viability was determined using the MTS assay. [b]IC_{50} is calculated using a dose-response non-linear regression.

The results are shown in the figures 1-5.

FIGURE 1: RP-UPLC-MS UV (λ = 306 nm) chromatograms of the activation of compound **30** (MTZ-γ-TZ, t_R 0.84 min) at a concentration of 125 µM in a 1.25mM solution of H_2O_2 (10 equiv.) in DMSO:PBS (1:1). Data points were collected after 0, 1, 4, 15, and 24 h by RP-UPLC-MS. Methotrexate elutes at t_R 0.62 min (identified with a commercially available reference sample). The peak at t_R 0.18 min corresponds to the solvent peak

FIGURE 2: MCF-7 *in vitro* cell viability assay incubated with methotrexate and compound **30** (MTZ-γ-TZ). Cells were incubated at increasing concentrations of tested compounds for 48 h before MTS reagent was added. Results are calculated as mean of triplicates (mean ± SD, n = 3), and IC_{50} is calculated using a dose-response non-linear regression. Pre-activation of

tested compounds with H_2O_2 was performed 24 h before experiment started as described in the experimental section.

FIGURE 3: NCI-H460 *in vitro* cell viability assay incubated with methotrexate and compound **30** (MTZ-γ-TZ). Cells were incubated at increasing concentrations of tested compounds for 48 h before MTS reagent was added. Results are calculated as mean of triplicates (mean ± SD, n = 3), and IC_{50} is calculated using a dose-response non-linear regression Pre-activation of tested compounds with H_2O_2 was performed 24 h before experiment started as described in the experimental section.

FIGURE 4: *In vitro* cell viability study of MCF-7 cells incubated for 48 h with 0.25, 0.062 and 0.015 µM concentrations of methotrexate and compound **30** (MTZ-γ-TZ) (mean ± SD, n = 3). Pre-activation of tested compounds with H_2O_2 was performed 24 h before experiment started as described in the experimental section.

FIGURE 5: *In vitro* cell viability study of NCI-H460 cells incubated for 48 h with 0.25, 0.062 and 0.015 µM concentrations of methotrexate and compound **30** (MTZ-γ-TZ) (mean ± SD, n = 3). Pre-activation of tested compounds with H_2O_2 was performed 24 h before experiment started as described in the experimental section.

EXAMPLE 5

In vivo animal assay

Materials and Methods

Animals: DBA/1J mice (male, 8-9 weeks) were obtained from Janvier, France. The mice were maintained in the animal house at Redoxis, Medicon Village, Lund, Sweden, where they were acclimatized for approximately one week before initiation of the experiment. All animal experiments were approved by the local animal ethic committee Malmö/Lund, Sweden, approved under the license N165-15.

*Induction of disease: collagen induced arthritis (*CIA) was induced by intradermal immunization with 100 µg of chicken type-II collagen (CII, Chondrex) in Complete Freund's Adjuvant (CFA, Difco) on day -1 *via* subcutaneous injection approximately 0.5 cm from the root of the tail. On day 21 a boost injection was administered in the same way with 50 µg CII. One week after the second immunization, onset of disease started to be observed (day 26).

Anti-arthritic effect of test compounds and health evaluation: mice were randomly divided in 5 groups (n = 8 per group): group I (vehicle), group II (**MTX**, Sigma Aldrich, 7.0 mg/kg, *i.p.*), group III (**30**, MTZ-γ-TZ, 8.3 mg/kg, *i.p.*). Vehicle and compound (2% DMSO in PBS, Life Technologies, injection volume 370 µL) were dosed daily intraperitoneally for 14 days, starting at onset of disease (day 27). Disease was evaluated three times per week in a blinded fashion, starting at day 18 until the end of the experiment (day 40). The reduction of swelling in the limbs was used as macroscopic score. A macroscopic scoring system of the four limbs ranging from 0 to 15 (1 point for each swollen or red toe) was used, meaning a maximal score of 60 per mice. For ethical reasons and restrictions, mice with score exceeding 45 were removed from the experiment. The general health of mice was evaluated three times per week after disease induction. As an indicator of general health, animal body weight was used.

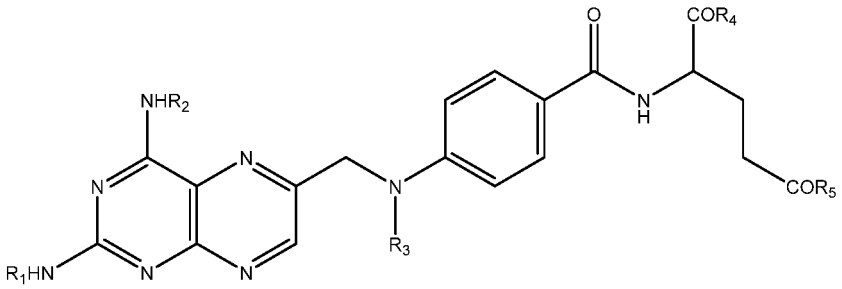
FIGURE 6 shows suppression of CIA development in mice during treatment with methotrexate (MTX), and prodrug **30** (MTZ-γ-TZ) (n = 8 per group). DBA/1J mice were given the indicated amounts of compound daily and disease progression vas evaluated three times per week starting on day 27. One animal in vehicle group was sacrificed pre-termination due to high score. Data represents mean values of arthritic score ± SEM. * represents a p-value <0.05 and ** represents a p-value <0.01 for comparison between MTX and vehicle, while † represents a p-value <0.05 for comparison between **30** and vehicle.

FIGURE 7 shows the general health of mice was evaluated three times per week during CIA as the average body weight in groups of animals (n = 8) tested with vehicle, MTX, and **30** (MTZ-γ-TZ). One animal in vehicle group was sacrificed pre-termination due to high score. Data represents mean values of arthritic score ± SEM.

ASPECTS OF THE INVENTION

ASPECT 1

A compound having the formula I

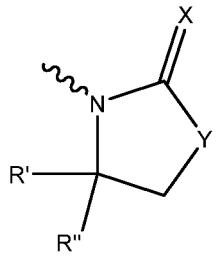


I

wherein R1 and R2 are hydrogen;

R3 is selected from the group consisting of hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, and C_2-C_6alkynyl;

R4 and R5 are selected from the group consisting of OH, O-C_{1-6}alkyl, O-C_{2-6}alkenyl, O-aryl, O-C_{1-6}alkyl-aryl, and a ring structure of formula II:



II

wherein X and Y are independently S or O, and R' and R'' are independently selected from hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, aryl, and C_{1-6}alkyl-aryl;

provided that at least one of R4 and R5 have the ring structure of formula II;

as well as pharmaceutically acceptable salts, solvates, and stereoisomers thereof.

ASPECT 2

The compound according to aspect 1, wherein Y is S.

ASPECT 3

The compound according to any one of the preceding aspects, wherein X is O.

ASPECT 4

The compound according to any one of the preceding aspects, wherein R' and R'' are both hydrogen.

ASPECT 5

The compound according to any one of the preceding aspects, wherein R3 is C_{1-6}alkyl, preferably C_{1-4}alkyl, preferably methyl or ethyl, more preferably methyl.

ASPECT 6

The compound according to any one of the preceding aspects, wherein R4 is 3-thiazolidinonyl and R5 is OH or O-C_{1-6}alkyl.

ASPECT 7

The compound according to any one of the preceding aspects, wherein R4 is OH or O-C_{1-6}alkyl, and R5 is 3-thiazolidinonyl.

ASPECT 8

The compound according to any one of the preceding aspects, wherein R4 and R5 are both 3-thiazolidinonyl.

ASPECT 9

The compound according to any one of the preceding aspects selected from the group consisting of:

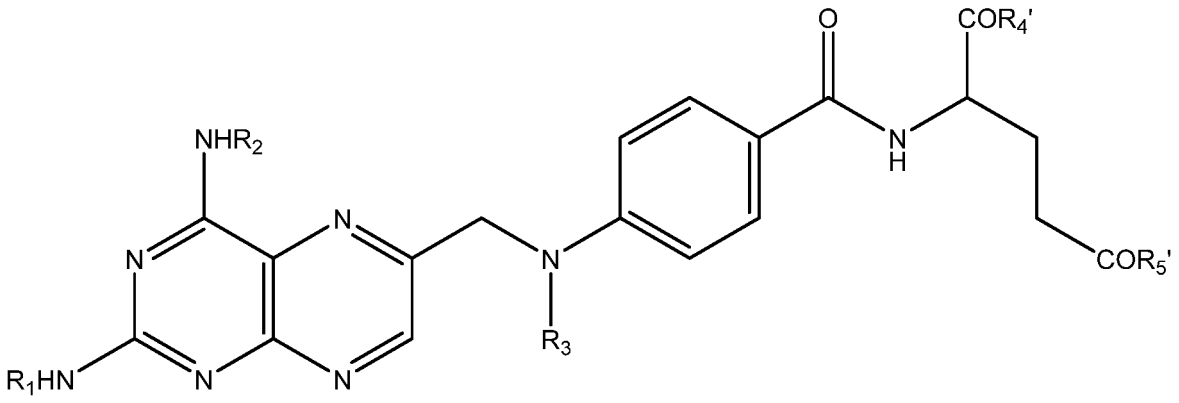
4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino)-N-(1,5-dioxo-1,5-bis(2-oxothiazolidin-3-yl)pentan-2-yl)benzamide (**28**),

tert-butyl (*S*)-2-(4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino)-benzamido)-5-oxo-5-(2-oxothiazolidin-3-yl)pentanoate (**67**), and

(*S*)-2-(4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino)benzamido)-5-oxo-5-(2-oxothiazolidin-3-yl)pentanoic acid (**30**).

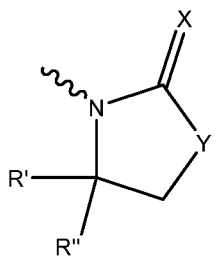
ASPECT 10

A method for the preparation of a compound of formula I, said method comprising the step of reacting a compound of formula IA,



IA

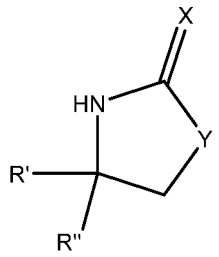
in which R1, R2, and R3 are as defined in any of claims 1-9; and R4' and R5' are selected from the group consisting of OH, O-C_{1-6}alkyl, O-C_{2-6}alkenyl, O-aryl, O-C_{1-6}alkyl-aryl, and a ring structure of formula II:



II

wherein X and Y are independently S or O, and R' and R'' are independently selected from hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, aryl and C_{1-6}alkyl-aryl; wherein at least one of R4' and R5' are OH;

with a compound of formula IIA:



IIA

in which X, Y, R' and R'' are as defined in any of aspects 1-9; in an amide coupling reaction.

ASPECT 11

A pharmaceutical composition comprising a compound according to any one of aspects 1-9, optionally in combination with one or more excipients.

ASPECT 12

A compound according to any one of aspects 1-9 for use as a prodrug for the treatment of inflammatory diseases or cancer, such as wherein said inflammatory disease is selected from the group consisting of rheumatoid arthritis (RA), juvenile dermatomyositis, psoriasis, psoriatic arthritis, lupus, sarcoidosis, atopic dermatitis, eczema, Crohn's disease, colitis ulcerosa, multiple sclerosis, and Amyotropic Lateral Sclerosis (ALS).

ASPECT 13

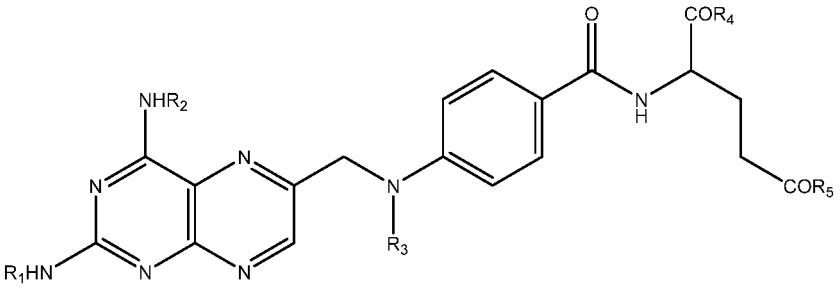
A method for the treatment of a patient suffering from inflammatory diseases or cancer, such as wherein said inflammatory disease is selected from the group consisting of rheumatoid arthritis (RA), juvenile dermatomyositis, psoriasis, psoriatic arthritis, lupus, sarcoidosis, atopic dermatitis, eczema, Crohn's disease, colitis ulcerosa, multiple sclerosis, and

Amyotropic Lateral Sclerosis (ALS), said method comprising administering a compound according to any one of aspects 1-9 to said patient.

5

Claims

1. A compound having the formula I

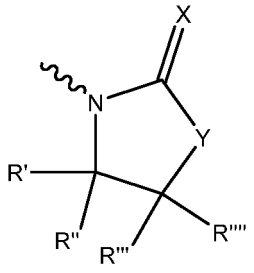


I

wherein R1 and R2 are hydrogen;

R3 is selected from the group consisting of hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, and C_2-C_6alkynyl;

R4 and R5 are selected from the group consisting of OH, O-C_{1-6}alkyl, O-C_{2-6}alkenyl, O-C_{6-12}aryl, O-C_{4-11}heteroaryl, O-C_{1-6}alkyl-C_{6-12}aryl, and a ring structure of formula II:

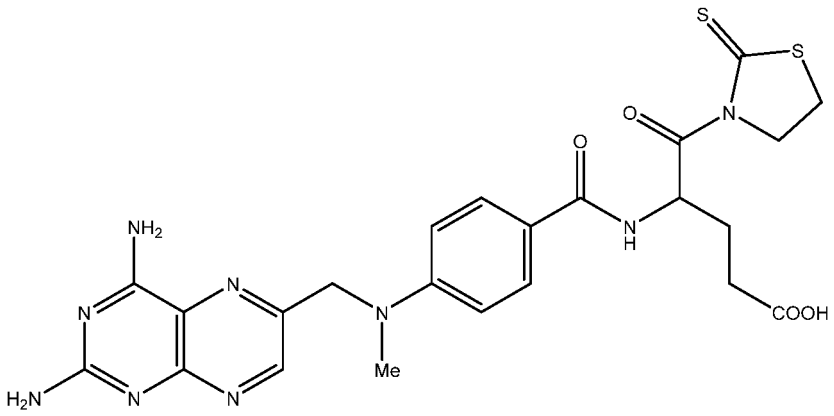


II

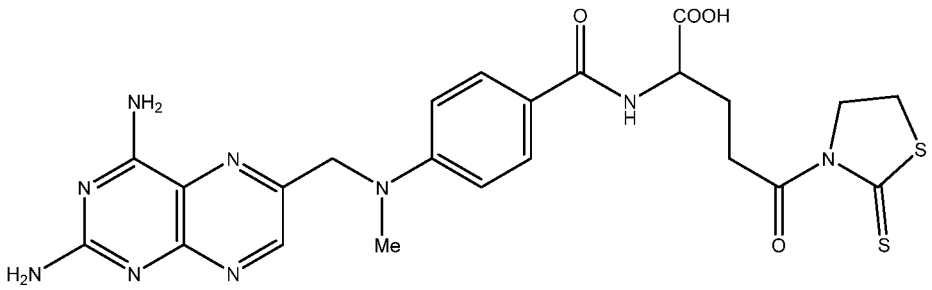
wherein X and Y are independently S or O, and R', R'', R''', and R'''' are independently selected from hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, C_{6-12}aryl, C_{4-11}heteroaryl and C_{1-6}alkyl-C_{6-12}aryl;

provided that at least one of R4 and R5 have the ring structure of formula II;

with the proviso that the compounds



and



are disclaimed;

as well as pharmaceutically acceptable salts, solvates, and stereoisomers thereof.

2. The compound according to claim 1, wherein Y is S.

3. The compound according to any one of the preceding claims, wherein X is O.

4. The compound according to any one of the preceding claims, wherein R',R'', R''', R'''' are hydrogen.

5. The compound according to any one of the preceding claims, wherein R3 is C_{1-6}alkyl, preferably C_{1-4}alkyl, preferably methyl or ethyl, more preferably methyl.

6. The compound according to any one of the preceding claims, wherein R4 is 3-thiazolidinonyl and R5 is OH or O-C_{1-6}alkyl.

7. The compound according to any one of the preceding claims, wherein R4 is OH or O-C_{1-6}alkyl, and R5 is 3-thiazolidinonyl.

8. The compound according to any one of the preceding claims, wherein R4 and R5 are both 3-thiazolidinonyl.

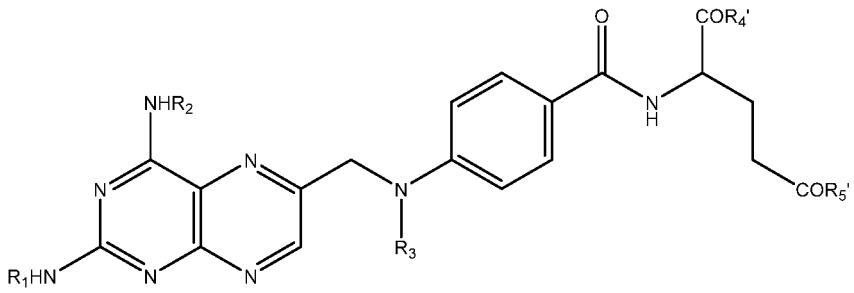
9. The compound according to any one of the preceding claims selected from the group consisting of:

4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino)-N-(1,5-dioxo-1,5-bis(2-oxothiazolidin-3-yl)pentan-2-yl)benzamide (**28**),

tert-butyl (*S*)-2-(4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino)-benzamido)-5-oxo-5-(2-oxothiazolidin-3-yl)pentanoate (**67**), and

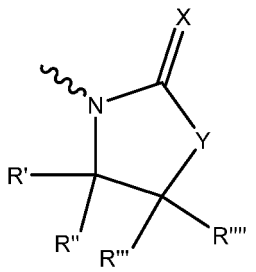
(*S*)-2-(4-(((2,4-diaminopteridin-6-yl)methyl)(methyl)amino)benzamido)-5-oxo-5-(2-oxothiazolidin-3-yl)pentanoic acid (**30**).

10. A method for the preparation of a compound of formula I, said method comprising the step of reacting a compound of formula IA,



IA

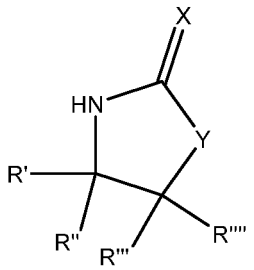
in which R1, R2, and R3 are as defined in any of claims 1-9; and R4' and R5' are selected from the group consisting of OH, O-C_{1-6}alkyl, O-C_{2-6}alkenyl, O-C_{6-12}aryl, O-C_{4-11}heteroaryl, O-C_{1-6}alkyl-C_{6-12}aryl, and a ring structure of formula II:



II

wherein X and Y are independently S or O, and R', R'', R''' and R'''' are independently selected from hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, C_{6-12}aryl, C_{4-11}heteroaryl and C_{1-6}alkyl-C_{6-12}aryl; wherein at least one of R4' and R5' are OH;

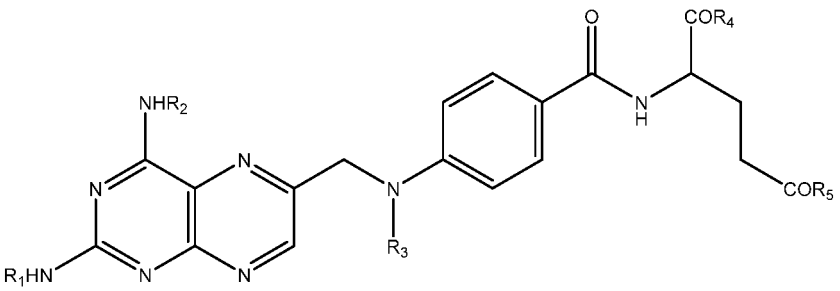
with a compound of formula IIA:



IIA

in which X, Y, R', R'', R''' and R'''' are as defined in any of claims 1-9; in an amide coupling reaction.

11. A pharmaceutical composition comprising a compound having the formula I

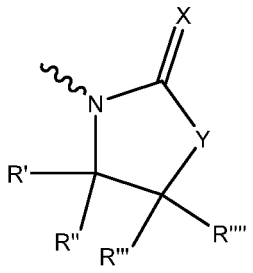


I

wherein R1 and R2 are hydrogen;

R3 is selected from the group consisting of hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, and C_2-C_6alkynyl;

R4 and R5 are selected from the group consisting of OH, O-C_{1-6}alkyl, O-C_{2-6}alkenyl, O-C_{6-12}aryl, O-C_{4-11}heteroaryl, O-C_{1-6}alkyl-C_{6-12}aryl, and a ring structure of formula II:



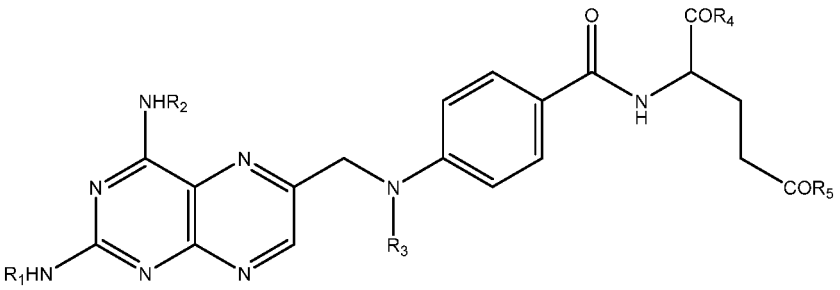
II

wherein X and Y are independently S or O, and R', R'', R''' and R'''' are independently selected from hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, C_{6-12}aryl, C_{4-11}heteroaryl and C_{1-6}alkyl-C_{6-12}aryl;

provided that at least one of R4 and R5 have the ring structure of formula II;

as well as pharmaceutically acceptable salts, solvates, and stereoisomers thereof; optionally in combination with one or more excipients.

12. A compound having the formula I

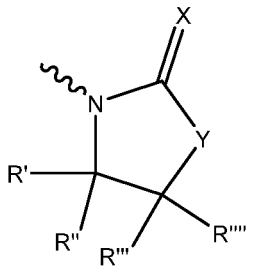


I

wherein R1 and R2 are hydrogen;

R3 is selected from the group consisting of hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, and C_2-C_6alkynyl;

R4 and R5 are selected from the group consisting of OH, O-C_{1-6}alkyl, O-C_{2-6}alkenyl, O-C_{6-12}aryl, O-C_{4-11}heteroaryl, O-C_{1-6}alkyl-C_{6-12}aryl, and a ring structure of formula II:



II

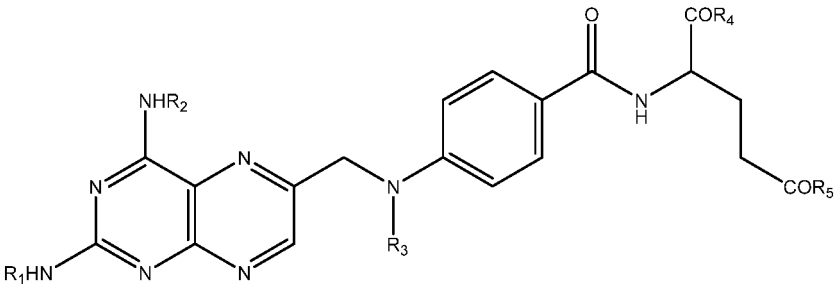
wherein X and Y are independently S or O, and R', R'', R''' and R'''' are independently selected from hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, C_{6-12}aryl, C_{4-11}heteroaryl and C_{1-6}alkyl-C_{6-12}aryl;

provided that at least one of R4 and R5 have the ring structure of formula II; as well as pharmaceutically acceptable salts, solvates, and stereoisomers thereof for use for the treatment of inflammatory diseases or cancer.

13. The compound according to claim 12, wherein said inflammatory disease is selected from the group consisting of rheumatoid arthritis (RA), juvenile dermatomyositis, juvenile rheumatoid arthritis, psoriasis, psoriatic arthritis, lupus, sarcoidosis, atopic dermatitis, eczema, Crohn's disease, uveitis associated with juvenile idiopathic arthritis or ulcerative colitis, colitis ulcerosa, multiple sclerosis, Amyotropic Lateral Sclerosis (ALS), non-infectious ocular inflammation, vasculitis, systemic lupus erythematosus, and eosinophilic fasciitis.

14. The compound according to claim 12, wherein said cancer is selected from the group consisting of acute lymphocytic leukemia, meningeal leukemia, myeloproliferative neoplasm, breast cancer, squamous cell carcinoma, lymphosarcoma, osteosarcoma, advanced mycosis fungoides (cutaneous T cell lymphoma), small cell types lung cancer, non-small cell lung cancer, and non-Hodgkin's lymphoma.

15. A method for the treatment of a patient suffering from inflammatory diseases or cancer, said method comprising administering a compound having the formula I

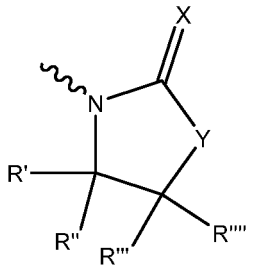


I

wherein R1 and R2 are hydrogen;

R3 is selected from the group consisting of hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, and C_2-C_6alkynyl;

R4 and R5 are selected from the group consisting of OH, O-C_{1-6}alkyl, O-C_{2-6}alkenyl, O-aryl, O-C_{1-6}alkyl-aryl, and a ring structure of formula II:



II

wherein X and Y are independently S or O, and R', R'', R''' and R'''' are independently selected from hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, aryl, and C_{1-6}alkyl-aryl; provided that at least one of R4 and R5 have the ring structure of formula II; as well as pharmaceutically acceptable salts, solvates, and stereoisomers thereof; to said patient.

16. The method according to claim 15, wherein said inflammatory disease is selected from the group consisting of rheumatoid arthritis (RA), juvenile dermatomyositis, juvenile rheumatoid arthritis, psoriasis, psoriatic arthritis, lupus, sarcoidosis, atopic dermatitis, eczema, Crohn's disease, uveitis associated with juvenile idiopathic arthritis or ulcerative colitis, colitis ulcerosa, multiple sclerosis, Amyotropic Lateral Sclerosis (ALS), non-infectious ocular inflammation, vasculitis, systemic lupus erythematosus, and eosinophilic fasciitis.

17. The method according to claim 15, wherein said cancer is selected from the group consisting of acute lymphocytic leukemia, meningeal leukemia, myeloproliferative neoplasm, breast cancer, squamous cell carcinoma, lymphosarcoma, osteosarcoma, advanced mycosis fungoides (cutaneous T cell lymphoma), small cell types lung cancer, non-small cell lung cancer, and non-Hodgkin's lymphoma.

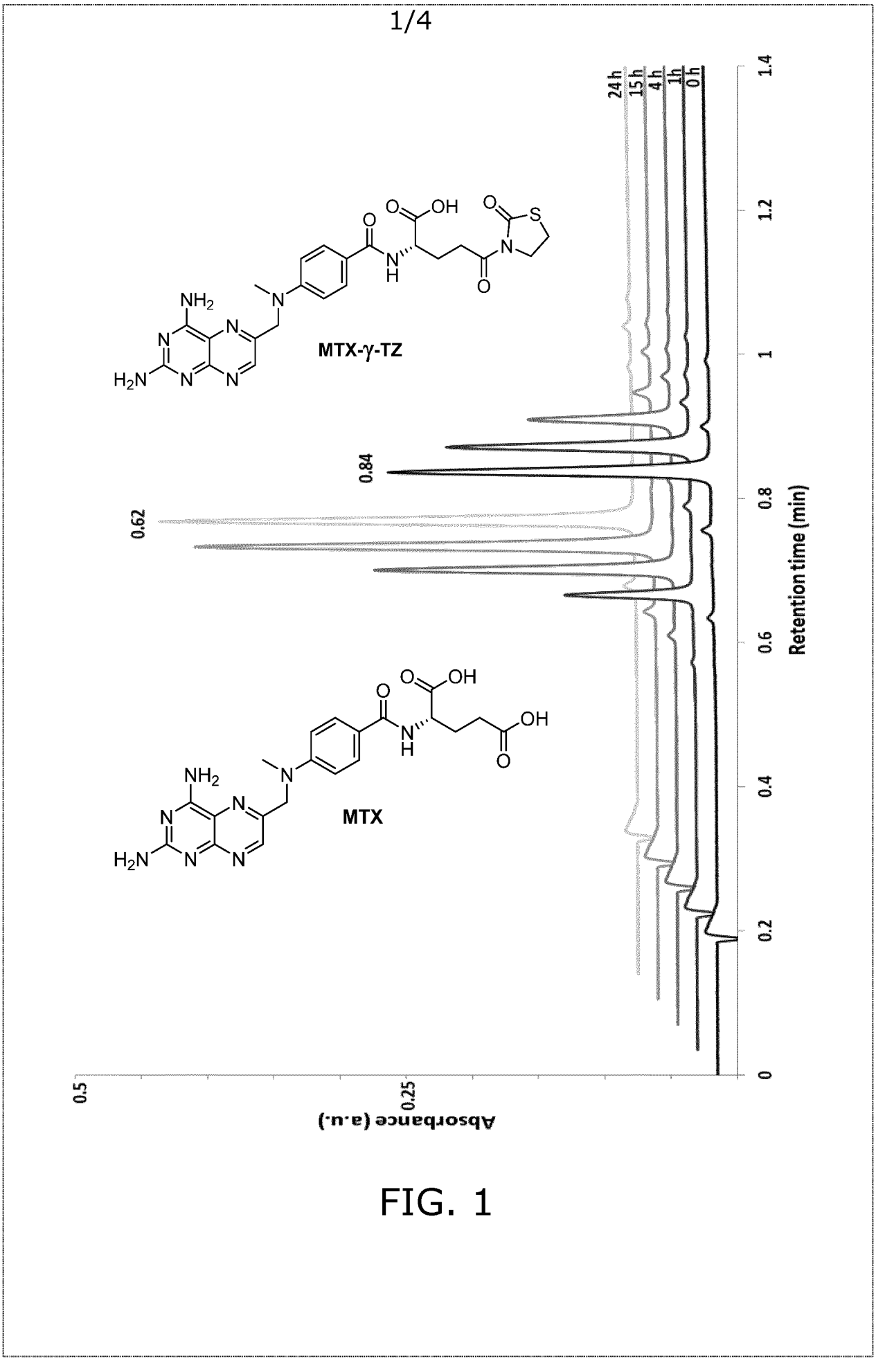

24 h
15 h
4 h
1 h
0 h
0.84
0.62
MTX-γ-TZ
MTX
Retention time (min)
Absorbance (a.u.)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
0.5
0.25

FIG. 1

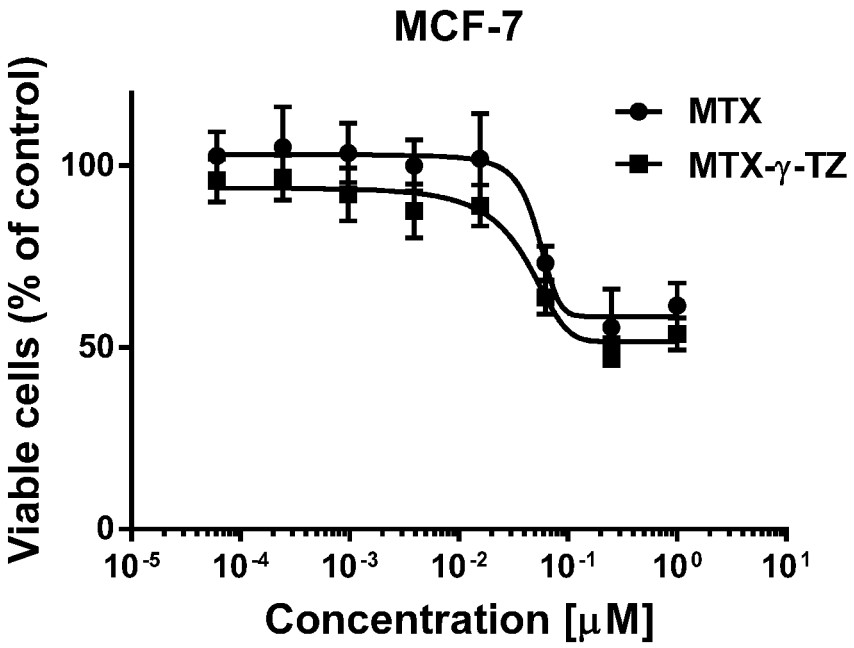

MCF-7
MTX
MTX-γ-TZ
Viable cells (% of control)
100
50
0
10-5
10-4
10-3
10-2
10-1
100
101
Concentration [μM]

FIG. 2

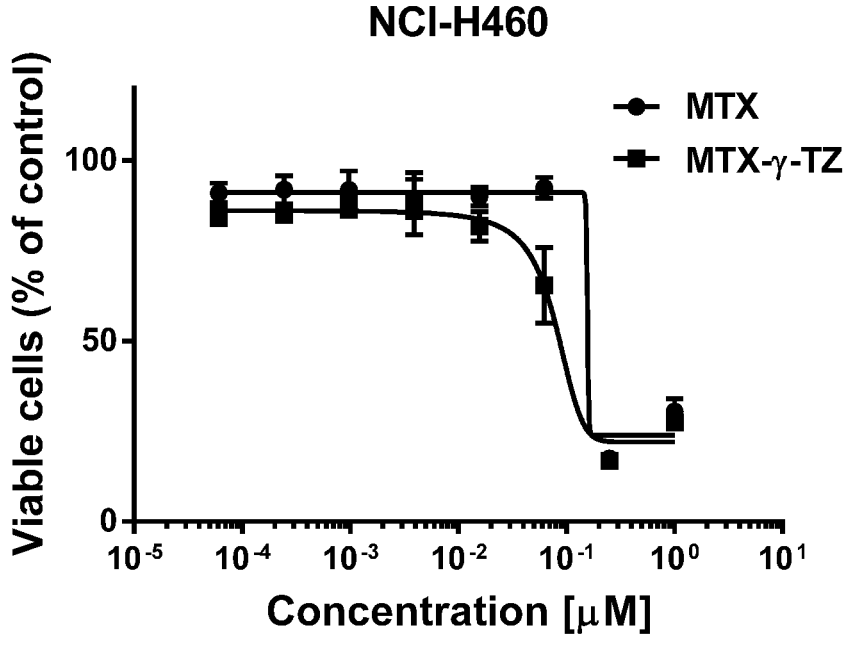

NCI-H460
MTX
MTX-γ-TZ
Viable cells (% of control)
100
50
0
10-5
10-4
10-3
10-2
10-1
100
101
Concentration [μM]

FIG. 3

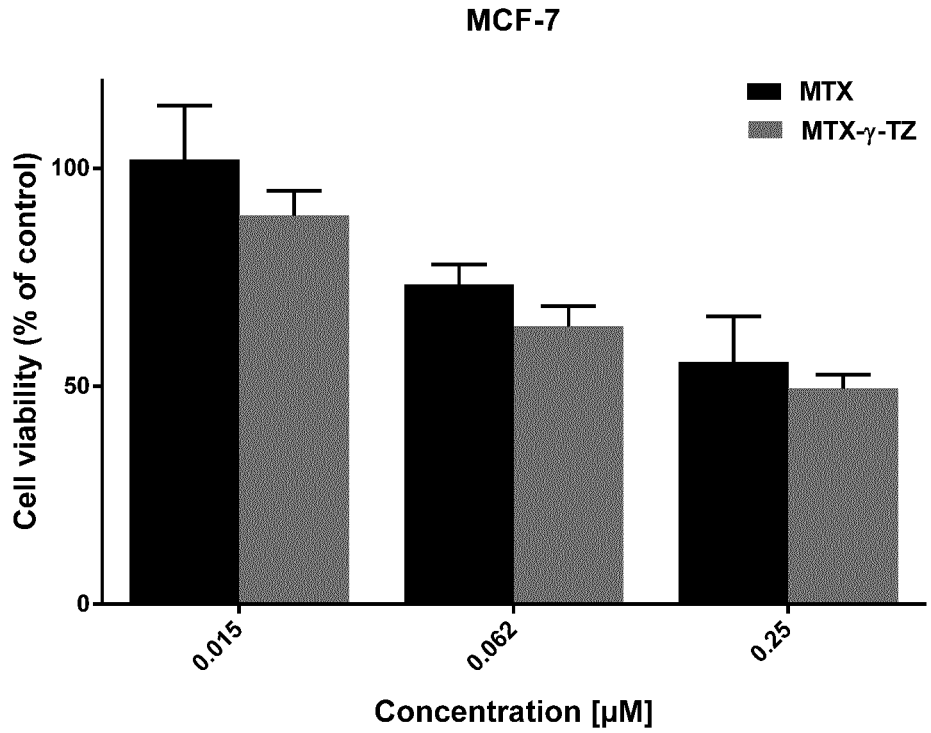

MCF-7
MTX
MTX-γ-TZ
Cell viability (% of control)
100
50
0
0.015
0.062
0.25
Concentration [μM]

FIG. 4

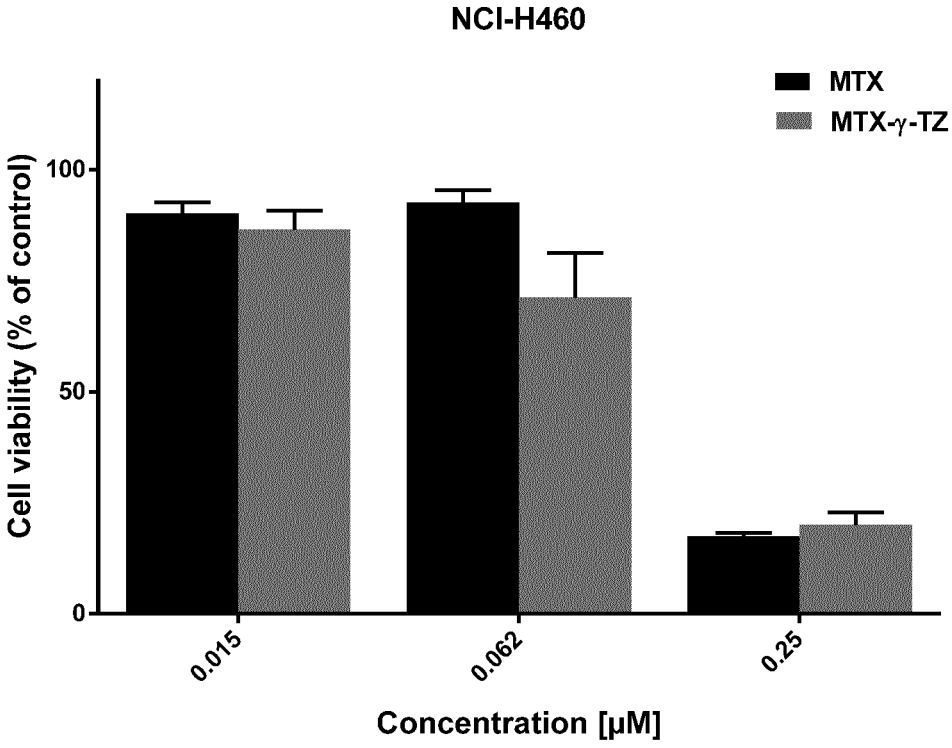

NCI-H460
MTX
MTX-γ-TZ
Cell viability (% of control)
100
50
0
0.015
0.062
0.25
Concentration [μM]

FIG. 5

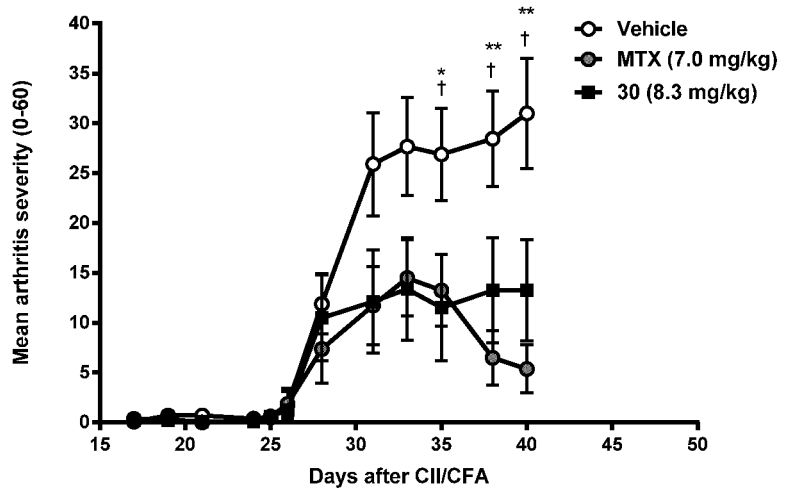

Mean arthritis severity (0-60)
Days after CII/CFA
Vehicle
MTX (7.0 mg/kg)
30 (8.3 mg/kg)
*
†
**
†
**
†

FIG. 6

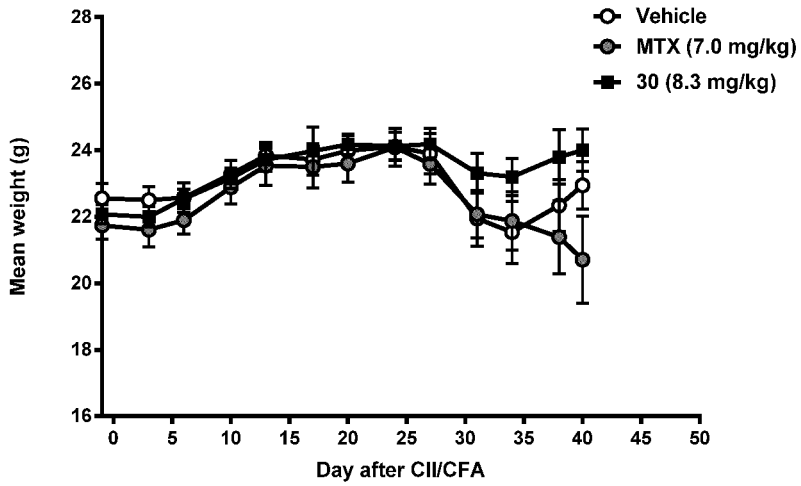

Mean weight (g)
Day after CII/CFA
Vehicle
MTX (7.0 mg/kg)
30 (8.3 mg/kg)

FIG. 7

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/071456

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D487/04 A61K31/519 A61P29/00 A61P35/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KHAN ET AL: "Methotrexate: a detailed review on drug delivery and clinical aspects", EXPERT OPINION ON DRUG DELIVERY,, vol. 9, 1 January 2012 (2012-01-01), pages 151-169, XP002762879, the whole document -----	1-17
A	US 2013/045949 A1 (PENG XIAOHUA [US] ET AL) 21 February 2013 (2013-02-21) the whole document -----	1-17

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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Date of the actual completion of the international search	Date of mailing of the international search report
15 September 2017	27/09/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Baston, Eckhard

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WEI WEN-HAO ET AL: "Gadolinium texaphyrin-methotrexate conjugates. Towards improved cancer chemotherapeutic agents", ORGANIC & BIOMOLECULAR CHEMISTRY, ROYAL SOCIETY OF CHEMISTRY, GB, vol. 3, no. 18, 21 September 2005 (2005-09-21), pages 3290-3296, XP002608176, ISSN: 1477-0520 compound 8 -----	1-17

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2017/071456

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2013045949 A1	21-02-2013	US 2013045949 A1	21-02-2013
		US 2014200250 A1	17-07-2014